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CONTENT OF THE DIET TO THE GROWTH AND
CHEMICAL COMPOSITION OF THE HAIR,
CHANGES IN THE CHEMICAL COMPOSITION
OF HAIR CAUSED BY EXTRA-
TION WITH WATER

By HOWARD DAVID LIGHTBODY

*A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
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THE METABOLISM OF SULFUR.

XV. THE RELATION OF THE PROTEIN AND CYSTINE CONTENT OF THE DIET TO THE GROWTH OF THE HAIR IN THE WHITE RAT.

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The use of laboratory animals as experimental subjects in studies in nutrition has led to suggestions, for the most part incidental, of a possible relationship of the protein content of the diet to the growth of the hair. Thus Abderhalden (1) noted that in one of his dogs, which had been clipped just prior to the beginning of the experiment, a full coat of hair was produced during the time in which the animal was fed a diet containing completely hydrolyzed protein as the sole source of nitrogen. McCollum, Simmonds, and Parsons (2) reported the loss of hair in white rats fed on diets containing a low percentage of protein, derived largely from potato. Boas (3) observed that the ingestion of diets in which a commercial preparation of egg white was the sole source of protein resulted in the loss of hair of white rats. This effect was not noted when fresh egg white was fed (4). It should be noted that improvement was obtained by the substitution of either 20 per cent casein or serum albumin for the proteins of the egg white. Hartwell (5) observed that loss of fur in white rats was closely correlated with the quality of the protein of the diet. However, since a favorable response was obtained with the addition to the diet of either the biologically adequate protein, casein, or the incomplete protein, gelatin, it is difficult to interpret these beneficial results as due solely to the variations in the amino acid make-up of the proteins.

That production of hair is related to the dietary intake of the

amino acid, cystine, which is present in notable amounts in hair and similar epidermal tissues (6), was suggested by Evvard, Dox, and Guernsey (7). The offspring of sows fed during pregnancy with "black albumen," a commercial protein concentrate derived from blood, were larger with heavier and darker coats of hair than the controls, whose mothers had received a diet derived from cereals chiefly. They suggested that since "a large proportion of the sulfur found in black albumen is supposedly present as cystine, it is not unreasonable to assume that the addition of black albumen furnishes the cystine, the basal constituent of hair growth."

In one rabbit on an experimental diet, the protein element of which was derived from wheat gluten and casein, Sjollesma (8) noted the appearance of bald spots, which upon the addition of 0.18 gm. of cystine to the ration daily, became covered with thick fur in 14 to 20 days, an effect which was "marvelous."

That cystine is importantly concerned in the growth of human hair has been maintained by Zuntz and coworkers (9), who used a commercial product (Humagsolan) prepared by the hydrolysis of keratin. The beneficial results in the increased growth of the hair were attributed to the presence of cystine in the material fed. Blaschko (10) was able to confirm the findings of Zuntz, the preparation being effective in cases of baldness, providing the papillæ had not been destroyed. Fuhs (11), however, was unable to obtain such marked beneficial results.

The data in support of the theory of the supposed relation of the protein or cystine of the food to the production of the hair have thus been derived from observations made incidentally during the conduct of other experiments or from studies in which the influence of the other possible variable factors has not been excluded. Factors of the diet, other than the protein or cystine content, have usually been varied, and there has been no satisfactory criterion of hair production or of a change in cystine metabolism.

It has been the purpose of the present series of experiments to study the production of hair in young white rats, when fed diets varying only in their protein content; *i.e.*, diets in which the amount of cystine has been shown to be the limiting factor. If in cases of an inadequate supply of cystine in the diet, the demand for cystine both for the growth of more essential tissues and also

for the elaboration of the less essential epidermal structure, the hair, cannot be met, will the demand for cystine for purposes of growth take precedence over the requirements for the production of hair?

This problem involves the more general question of the competition between a more essential and a less essential tissue for a unit necessary for each. The high content of the hair in cystine and the possibility of actually measuring the growth of this structure under varying conditions of nutrition make this tissue almost ideal for the study of this problem of competition for an essential chemical constituent.

EXPERIMENTAL.

White rats at the age of 30 days were selected from our colony for these studies in such a manner that litter mates were distributed between the several diets chosen, thus eliminating as far as possible variation between litters. Because of the difficulty in obtaining the necessary number of animals in a sufficiently short time to eliminate any possible seasonal differences, it was necessary to extend the period of the experiments over 6 months from May to November. Fourteen animals were placed in each of the experimental groups, nine of each group being killed at the end of 11 weeks. In order to determine whether white rats under laboratory conditions formed a winter coat of a character different from that formed in spring or summer, the remaining five animals of each group were continued on the experimental diets for an additional 6 weeks. As no clear evidence of the formation of such a coat was obtained, the two periods are not differentiated in the summary tables given later.

The diets chosen were based upon the experimental data of Sherman and Woods (12), which have been confirmed repeatedly in our own laboratory (13). These investigators were able to demonstrate that the growth of white rats on a basal diet whose protein was derived from whole milk powder (16.7 per cent) was limited by the cystine content of the diet and that improved rates of growth could be obtained in proportion to the amount of cystine or casein added as a supplement to the basal diet. The basal diet (Diet A) used in our experiments, together with the modifications designed to increase the cystine content through the

addition of varying amounts of casein, is presented in Table I. It will be noted that when casein was introduced into the diet, it replaced an equal amount of dextrin, the calorific value thus remaining approximately the same for all the diets.

As controls, four representative groups from our colony were used for the collection of hair. In order to secure information as to the hair production prior to the beginning of the experimental periods, one group (Group V) was killed at the age of 30 days and the amount of hair which had been produced at that age was determined. A second control group (Group VI) received an adequate mixed diet of ground biscuit (50 per cent), powdered whole milk (20 per cent), wheat germ (20 per cent), and casein

TABLE I.
Experimental Diets.

Diet.....	A	B	C	D	E
Whole milk powder.....	16.70	16.70	16.70	16.70	16.70
Dextrin.....	79.00	78.53	77.00	71.00	63.00
Cod liver oil.....	1.00	1.00	1.00	1.00	1.00
Salt mixture*.....	1.65	1.65	1.65	1.65	1.65
Dried yeast.....	1.65	1.65	1.65	1.65	1.65
Casein.....		0.47	2.00	8.00	16.00

*Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, **37**, 557 (1919).

(10 per cent) during the same periods of time as the animals which were fed the diets in which the cystine content was the limiting factor. It was observed early in the course of the experiments that at the end of the 11 week period, the rats which received the least amount of casein and cystine (Group I, Diet B) had reached an average body weight of about 120 gm. As a further control on this group, a third series (Group VII) was fed the same stock diet as Group VI, fully adequate for normal growth, but was killed for a study of the amount of hair produced, when the animals had attained a body weight of approximately 120 gm. This stage in development was reached when the animals were from 6 to 7 weeks old; *i.e.*, after a period of 2 to 3 weeks on the stock diet. A fourth control group (Group VIII) was composed of adult females varying in age from 12 to 14 months.

For the collection of the hair, the pelts were removed immediately after the sacrifice of the animals by cutting on a medial line of the ventral surface, about the legs at the tarsal bones, and about the neck on a line immediately posterior to the ears. It was thus possible to remove practically all of the hide except the small portions on the ears and fore part of the head and the extreme ends of the limbs. The pelts were immediately scraped free from as much subcutaneous tissue as possible and weighed while still moist. Each pelt was then immersed in a liter of phosphate solution buffered at a pH of 6.8 to which 1 gm. of a commercial pancreatin preparation was added. After incubation at 37° for 24 hours, the pelts were removed from the digestion mixture and the hair, loosened in the digestion, was separated from the other tissue. In order to remove adhering tissue, the hair was suspended repeatedly in distilled water, and the mixture was stirred and filtered through 60 mesh copper gauze. The hair was then transferred to weighed paper extraction thimbles; the thimbles were allowed to drain and then dried for 24 hours at 60° in a vacuum oven. The thimbles were then transferred to extraction apparatus of the Bailey-Walker type and the hair was extracted for periods of 24 hours each with absolute alcohol, ether, and distilled water. The thimbles were again dried *in vacuo* at 60° and stored in a desiccator over calcium chloride. For the weighing of the thimbles and hair, the thimbles were placed in thin glass-stoppered weighing bottles.

The animals were weighed weekly throughout the experiments. Body surface was calculated by the formula of Carman and Mitchell (14). From the data obtained were calculated the ratios of the weight of the hair to body weight and to body surface, which are expressed in Tables III to VII as mg. of hair per gm. of body weight or per sq. cm. of body surface.

DISCUSSION.

The average final weights of each group of animals are presented in Table II. It will be observed that, in confirmation of the work of Sherman and Woods (12), a significant change in the rate of growth occurred on the addition of increasing amounts of casein to the diet. Thus the animals of Group I, to whose diet a very small amount of casein (0.47 per cent) was added, attained

Growth of Hair

TABLE II.
Average Final Weight of Rats.

Group No.	Diet.	Age 15 wks. Weight.		Age 21 wks. Weight.		Group No.	Diet.	Weight.	
		Males.	Fe-males.	Males.	Fe-males.			Males.	Fe-males.
		gm.	gm.	gm.	gm.			gm.	gm.
I	B	124.7	101.4	144.9	143.4	V	Stock.	57.7	63.1
II	C	161.4	147.9	203.5	155.3		(Rats 30 days old.)		
III	D	249.9	154.7	270.0	175.1	VII	Stock.	122.5	113.7
IV	E	270.5	183.5	297.7	178.0		(Rats 6 to 7 wks. old.)		
VI	Stock.	297.1	158.1	312.3	193.6	VIII	Stock. (Adults.)		229.3

TABLE III.
Group V.

Young rats, 30 days old.

Rat No.	Sex.	Weight.	Surface.	Hair.	Hair.	
					Per gm. body weight.	Per sq. cm. body surface.
		gm.	sq. cm.	gm.	mg.	mg.
93	F.	67.4	188.1	0.4000	5.93	2.12
96	"	61.5	177.0	0.3220	5.23	1.81
97	"	70.5	193.8	0.5876	8.33	3.03
101	"	53.2	160.6	0.3836	7.21	2.38
92	M.	56.2	166.6	0.2908	5.17	1.74
94	"	59.9	173.9	0.4122	6.88	2.37
95	"	51.8	157.8	0.2461	4.75	1.55
98	"	51.1	156.4	0.5108	9.99	3.26
99	"	62.5	178.8	0.4680	7.48	2.61
100	"	64.7	183.0	0.5220	8.06	2.85
Average.....					6.90	2.37

TABLE IV.

Group VII. Control Diet.

Rats killed at average weight of 120 gm.; age 6 to 7 weeks.

Rat No.	Sex.	Weight.	Surface.	Hair.	Hair.	
					Per gm. body weight.	Per sq. cm. body sur- face.
		<i>gm.</i>	<i>sq. cm.</i>	<i>gm.</i>	<i>mg.</i>	<i>mg.</i>
109	F.	119.4	275.4	1.7606	14.74	6.39
111	"	106.9	255.8	1.1391	10.65	4.45
112	"	109.4	259.8	1.2281	11.22	4.72
113	"	120.4	276.9	1.3289	11.03	4.79
116	"	115.3	269.1	1.2114	10.50	4.50
117	"	111.3	262.8	1.1989	10.77	4.56
110	M.	118.2	273.6	1.5002	12.69	5.48
114	"	127.4	287.6	1.6096	12.63	5.59
115	"	122.1	279.5	1.2340	10.10	4.41
Average.....					11.59	4.98

TABLE V.

Group I, Diet B. Inadequate Content of Cystine.

[illegible]

average weights of only 124.7 (males) and 101.4 (females) gm. in a period of 11 weeks on the experimental diet, while as the amount of casein supplementing the basal diet was increased to 2, 8, and 16 per cent, the final body weights were 161.4, 249.9, and 270.5 gm. respectively for the males and 147.9, 154.7, and 183.5 gm. for the females. A comparison of the weights of Groups I and VII will show that the animals of the latter group were able to

TABLE VI.
Group IV, Diet E. Adequate Protein and Cystine Content.

Rat No.	Sex.	Age.	Weight.	Surface.	Hair.	Hair.	
						Per gm. body weight.	Per sq. cm. body surface.
		<i>wks.</i>	<i>gm.</i>	<i>sq. cm.</i>	<i>gm.</i>	<i>mg.</i>	<i>mg.</i>
64	F.	15	188.0	372.8	1.8240	9.70	4.89
68	"	15	175.5	356.1	2.1815	12.43	6.12
69	"	15	177.2	358.3	2.0180	11.38	5.63
70	"	15	193.5	380.0	2.2212	11.47	5.84
74	"	21	176.2	357.0	1.9235	10.91	5.38
75	"	21	179.2	361.0	2.2270	12.42	6.16
76	"	21	169.1	347.3	2.0487	12.11	5.89
77	"	21	187.8	372.5	2.8315	15.07	7.60
65	M.	15	213.0	404.3	2.5055	11.76	6.18
66	"	15	252.6	453.9	3.6814	14.57	8.11
67	"	15	241.6	430.6	2.9764	12.31	6.75
71	"	15	336.6	549.7	4.7230	14.03	8.59
72	"	15	309.0	519.2	3.9308	12.72	7.57
73	"	21	297.7	506.4	4.2160	14.16	8.32
Average.....						12.50	6.64

make as great increments in body weight in 2 to 3 weeks as did the rats on the inadequate low protein cystine-poor diet in the longer period of 11 weeks. It will be also observed that the animals which received the highest percentage of casein as a supplement to the basal diet (Group IV, Diet E) were able to grow practically as rapidly as the animals maintained on the stock diet throughout the experimental period.

Detailed data are given for the young rats (Table III), the stock rats killed at a body weight of 120 gm. (Table IV), and the two

TABLE VII.
Influence of Diet on Production of Hair.

	Hair from:					
	Males.		Females.		All animals.	
	Per gm. body weight.	Per sq. cm. body surface.	Per gm. body weight.	Per sq. cm. body surface.	Per gm. body weight.	Per sq. cm. body surface.

Group I, Diet B.

	mg.	mg.	mg.	mg.	mg.	mg.
Minimum.....	3.20	1.51	4.50	1.90		
Maximum.....	6.90	3.17	8.84	4.14		
Average.....	5.44	2.41	5.98	2.58	5.70	2.49
No. of rats.....	7	7	7	7	14	14

Group II, Diet C.

	mg.	mg.	mg.	mg.	mg.	mg.
Minimum.....	6.02	2.73	6.20	2.89		
Maximum.....	10.93	5.54	8.82	4.08		
Average.....	7.54	3.69	7.67	3.59	7.60	3.64
No. of rats.....	7	7	7	7	14	14

Group III, Diet D.

	mg.	mg.	mg.	mg.	mg.	mg.
Minimum.....	11.73	6.39	9.10	4.23		
Maximum.....	16.78	9.55	12.15	6.10		
Average.....	13.58	7.61	10.92	5.24	11.49	5.77
No. of rats.....	3	3	11	11	14	14

Group IV, Diet E.

	mg.	mg.	mg.	mg.	mg.	mg.
Minimum.....	11.76	6.18	9.70	4.89		
Maximum.....	14.57	8.59	15.07	7.60		
Average.....	13.25	7.58	11.93	5.93	12.50	6.64
No. of rats.....	6	6	8	8	14	14

Group V, 30 days old.

	mg.	mg.	mg.	mg.	mg.	mg.
Minimum.....	4.75	1.57	5.23	1.81		
Maximum.....	9.99	3.26	8.33	3.03		
Average.....	7.05	2.39	6.67	2.33	6.90	2.37
No. of rats.....	6	6	4	4	10	10

TABLE VII—*Concluded.*

	Hair from:					
	Males.		Females.		All animals.	
	Per gm. body weight.	Per sq. cm. body surface.	Per gm. body weight.	Per sq. cm. body surface.	Per gm. body weight.	Per sq. cm. body surface.
Group VI, stock diet.						
Minimum.....	14.21	7.90	9.59	4.71		
Maximum.....	21.08	13.11	14.30	7.05		
Average.....	16.40	9.71	12.51	6.12	14.18	7.66
No. of rats.....	6	6	8	8	14	14
Group VII, stock diet; 6 to 7 wks. old.						
Minimum.....	10.10	4.41	10.50	4.45		
Maximum.....	12.69	5.59	14.74	6.39		
Average.....	11.80	5.16	11.48	4.90	11.59	4.98
No. of rats.....	3	3	6	6	9	9
Group VIII, adult females.						
Minimum.....			8.04	4.47		
Maximum.....			12.07	6.42		
Average.....			10.28	5.51		
No. of rats.....			7	7		

experimental groups which received the least and greatest percentage of casein as a supplement to the basal diet (Tables V and VI). These groups we consider the most significant in the interpretation of our results. Since the animals which received diets containing intermediate amounts of casein and the two remaining control groups showed similar variations, it is considered sufficient to include these groups in the table in which the values for all the experimental animals are summarized (Table VII). The variations between the individual animals of the groups presented in the detailed tables are typical of the degree of variation observed in all the experiments.

As detailed in Table III, young white rats, killed at the age at which our feeding experiments with the other groups were begun,

had produced an amount of hair equivalent to 6.89 mg. per gm. of body weight or to 2.37 mg. per sq. cm. of body surface. These figures may be regarded as typical of the first or puppy coat of hair developed. According to Greenman and Duhring (15), this coat is succeeded by the adult type of hair which is heavier and slightly yellow tinted, shortly after the age of 6 weeks. This change in the character of the hair at the age of 6 or 7 weeks is shown in Table IV, in which the results with rats maintained on the stock diet until they have reached a weight of 120 gm. (age 6 to 7 weeks) are presented. These rats which are in the period of the active production of the adult coat of hair have elaborated a coat of hair whose weight in proportion to the body weight or body surface is *nearly double* that of the young rats of Group V (Table III). That the character of this coat is similar to that of older animals is shown by a comparison with the data for Group VI (Table VII). This latter group was fed the stock diet until the animals approached maturity and had passed through the period of manufacture of an adult coat. The amount of hair in proportion to body weight or surface was somewhat greater than with the animals of Group VII, but the difference was not as marked as the difference between Groups V and VII. It will also be noted that animals which received in the diet amounts of protein sufficient to produce normal or nearly normal rates of growth have also produced similar coats of hair, the heavier adult type (Group IV, Table VI; Groups III and IV, Table VII). From the above considerations, there would appear to exist two types of coats, a light puppy coat produced first in youth and a second heavier coat, which begins to be formed normally at about the 6th or 7th week of life and which in relation to the body weight or surface of the animal remains nearly the same throughout the later period of life, at least as far as our measurements demonstrate.

The animals of Group I (Table V), which received a diet inadequate in its protein (and cystine) content and which were able during a period of 11 weeks to make approximately the same gain in weight as the control animals (Group VII) on a stock diet in 2 to 3 weeks, did not produce a coat of hair of the *type grown by normal animals of either the same weight (Group VII) or age (Group VI)*. The average weight of the hair was 5.7 mg. per gm. of body weight, a figure more nearly comparable to the corre-

sponding weight of hair of the young rats at the age of 30 days (Group V), 6.8 mg., than to the figure 11.59 for normal rats of the same weight or 14.18 for normal rats of the same age. The rats of Group II, which also received a diet whose content of protein (and cystine) was inadequate for a normal rate of growth, although they were able to produce slightly heavier coats of hair (Table VII) than did the rats of Group I, did not show a coat of the "adult" type, such as that produced by animals of Groups III and IV (Table VII) maintained on diets satisfactory in their content of protein. It would thus appear that when the protein (and cystine) content of the diet is not adequate for both general somatic development and hair production, the demands for the production of the less essential biological structure, the hair, are not met and a coat of hair normal for an animal of that particular age or weight cannot be produced.

Further evidence in support of the theory that the demands for cystine for growth and body development take precedence over the requirement for the production of hair is afforded by the data obtained with Group VIII (Table VII), adult females¹ of 12 to 14 months of age. These animals were breeding stock which, as is usual in rat colonies, had successfully completed a series of cycles of pregnancy and lactation with short time intervals between each. In these animals the demand for protein for the building of fetal tissue and for milk production must have been great. If our hypothesis is correct, in such animals opportunity for replacement of hair lost through shedding should have been limited owing to the demands for cystine for the other purposes mentioned. In these groups the amount of hair was somewhat less than that present in the pelts of any other adult groups except those groups (Nos. I and II) in which the dietary supply of protein (and cystine) was known to be inadequate.

It is, of course, impossible to determine the amount of the hair shed by the animals during the period of our experiments. The figures given represent merely the weight of hair present in the pelt at the time the animals were sacrificed. It was noted that all the experimental groups shed hair during the period of study

¹ No sexual difference in the rate of the growth of hair was evident in the series of experimental animals. The data for the animals are summarized according to sex, however, in Table VII.

except Groups I and II on diets inadequate in protein content. These animals lost little hair and were apparently able to add but little to the total hair present when the experiment was started.

Further studies have indicated that not only the amount of hair produced but also the chemical composition of the hair was influenced by the protein (and cystine) content of the diet. These results will be presented in a later publication.

SUMMARY.

1. The production of the cystine-rich epidermal tissue, the hair, has been studied in young white rats fed diets in which the low cystine content of the protein fed (casein) was the chief factor limiting the rate of growth.

2. The amount of hair produced, like the general somatic development, was related to the protein (and cystine) content of the diet, but under the experimental conditions of the present study, the demands for protein (and cystine) for the growth of the hair appeared to be secondary in importance to the demands for growth of the body with its more essential tissues.

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THE METABOLISM OF SULFUR.

XVI. DIETARY FACTORS IN RELATION TO THE CHEMICAL COMPOSITION OF THE HAIR OF THE YOUNG WHITE RAT.

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Notable in the chemical composition of the proteins of the hair (and other epidermal structures) are the high contents of cystine and sulfur (1). In a previous study (2), we have been able to demonstrate that, in the young white rat, *the amount of hair produced was related to the protein (and cystine) content of the diet*, but that the demands for protein (and cystine) for the growth of the hair appeared to be secondary in importance to the demands for growth of the body with its more essential tissues.

In continuation of these studies, the present investigation is concerned with the *composition of the hair as related to dietary factors*. Are the amounts of cystine and sulfur present in hair influenced by factors of age or diet? Is the chemical composition of the protein of a tissue constant, or does the composition of a protein elaborated under conditions of a shortage of a specific amino acid reflect the lack of that particular amino acid in the diet? Since the hair is notably high in its content of cystine and since it has been demonstrated that cystine is used preferentially for general somatic development rather than for the growth of the hair under conditions of inadequate protein (and cystine) content of the diet (2), it has seemed that an attempt to solve this general question of the constancy of the composition of a tissue protein might well be made by a study of the composition of the hair produced by the young white rat on diets of varying protein and cystine content.

The general plan of the experiments was similar to that previously reported (2). Young white rats at the age of 30 days were selected from our colony in such a way that litter mates were distributed between the diets chosen, in order to eliminate so far as possible variations between litters. The animals were weighed weekly during an experimental period of 10 weeks. At the end of the 10 week period, the hair was collected by clipping. The samples were extracted with absolute alcohol and ether for periods of 24 hours each, dried at 60° *in vacuo*, and stored until analyzed in a desiccator over calcium chloride. Total sulfur was determined gravimetrically after oxidation of the sample in the Parr bomb; cystine, by the colorimetric method of Folin and Looney; and total nitrogen, by the Scales and Harrison modification of the Kjeldahl method (3). Duplicate analyses were carried out, when the amount of material available permitted; with the smaller animals, however, this was often impossible.

For the basis of the diets in which the amount of cystine present was the limiting factor, the milk powder-starch diet of Sherman and Woods (4) was used, modified as in previous studies (2). Groups 9 and 10 received diets in which 0.47 and 16 per cent respectively of casein replaced equivalent amounts of dextrin. In order to demonstrate more clearly that we were concerned with the quantity of cystine and not with the quantity of protein as such in these diets, the animals of Group 12 received a diet in which 6 per cent casein and 0.25 per cent of cystine replaced an equivalent amount of dextrin. The diets of Groups 10 and 12 should have contained approximately equal amounts of cystine,¹ although differing considerably in their content of total protein (casein).

As further controls, collections of hair were also made from rats fed the stock diet previously described (2) for 10 weeks (Group 11), from rats at the age of 30 days (Group 15), from animals fed

¹ Sherman and Woods (4) in their biological test of the cystine content of casein found that 1 gm. of casein was equivalent in its effect on growth to 0.025 gm. of cystine. Hence the addition of 0.25 per cent of cystine to 6 per cent casein (Group 12) should give a cystine content equivalent in *biological value* to 16 per cent casein (Group 10). On the basis of the *chemical analyses* (5), however, in which casein was found to contain only 0.25 per cent of cystine, the diet of Group 12 would have included much more cystine than the diet of Group 10.

TABLE I.
Composition of Hair.

Group No.	Diet.	Final weight, average.	Composition of hair of individual animals, per cent.	Average for group.
9	Casein, 0.47 per cent.	gm. 106.5	S. 3.80, 3.86, 3.98, 4.21, 4.03, 3.74, 3.74 Cystine. 10.96, 10.82, 11.79, 11.99, 11.47, 11.36, 11.50 N. 15.8, 16.0, 17.2, 17.0, 16.9	3.90 11.41
10	Casein, 16.0 per cent.	203.9	S. 4.27, 4.58, 4.31, 4.22, 4.43, 4.21, 4.68 Cystine. 12.36, 12.61, 12.89, 12.68, 12.99, 13.02, 12.79 N. 16.3, 16.1, 15.5, 16.2, 16.2, 16.9, 16.4	4.39 12.76
11	Stock diet.	199.5	S. 4.58, 4.28, 4.11, 3.89, 3.93, 4.64, 4.96 Cystine. 12.83, 12.68, 12.99, 12.27, 12.88, 12.52, 13.02 N. 15.5, 15.9, 15.2, 15.9, 15.7, 15.9, 16.1	4.34 12.74
12	Casein, 6.0 per cent. Cystine, 0.25 per cent.	181.7	S. 4.47, 4.59, 4.21, 4.30, 4.25, 4.52, 4.41, 4.34, 4.97, 4.73 Cystine. 12.48, 13.15, 12.78, 12.01, 12.24, 12.82, 12.87, 12.02, 11.82, 13.75	4.48 12.59
13	Low lysine.	124.9	S. 4.21, 3.98, 4.53, 4.50, 3.89, 4.96, 3.98, 4.15, 4.88 Cystine. 12.70, 12.46, 12.75, 12.88, 12.75, 12.40, 12.23, 12.29, 12.29, 12.77	4.34 12.59
14	Average weight, 120 gm.	118.9	S. 3.87, 3.96, 3.43, 3.22, 3.55, 3.37, 4.24 Cystine. 10.01, 10.80, 10.82, 11.01, 10.95, 11.67, 10.97, 10.90 N. 16.6, 17.0, 16.6, 16.8, 16.9, 16.6, 16.6, 16.4	3.66 10.98

TABLE I—*Concluded.*

Group No.	Diet.	Final weight, average.	Composition of hair, of individual animals, per cent.	Average for group.
15	Age, 30 days.	65.5 <i>gm.</i>	S. 3.72, 3.45, 3.28, 3.76, 3.93, 4.04, 4.02, 3.88 Cystine. 11.23, 11.02, 10.19, 10.19, 10.24, 10.37, 10.00, 11.32, 10.16 N. 17.0, 16.8, 16.3, 17.1, 16.7, 17.0, 17.2, 16.7	3.76 10.52
16	Adults.	281.8	S. 4.09, 4.60, 4.67, 4.32, 4.78, 4.46, 4.63, 4.59, 4.57, 4.36, 4.82 Cystine. 11.73, 12.21, 13.09, 13.68, 13.58, 14.63, 12.16, 12.62, 12.25, 12.05, 12.86 N. 15.6, 16.1, 16.0, 16.6, 16.5, 15.9, 15.8	4.53 12.80

the stock diet (for a period of 2 to 4 weeks) until they reached an approximate body weight of 120 gm. (Group 14), and from a group of stock adult rats (Group 16). In order to further control our experiments with diets varying in their content of cystine, hair was collected for analysis from a group of animals (Group 13) which were fed a diet which retarded growth as did the cystine-deficient diet, but which was inadequate in respect to a different amino acid, lysine. The lysine-deficient diet (6), upon which these animals were maintained for a period of 10 weeks was made up of rolled oats (60.0 per cent), dextrin (30.3 per cent), salt mixture (4.7 per cent), and butter fat (5.0 per cent). Animals on this diet showed a retarded growth similar to that of the animals of Group 9, which received a diet in which cystine was the factor limiting the rate of growth.

As shown in Table I, the analytical data fall into two general groups. All of the animals fed a diet adequate in its cystine content over an experimental period of 10 weeks had produced hair which contained nearly the same percentage of cystine (Groups 10 to 13), a figure which was comparable also to the cystine content of the hair of the adult animals (Group 16) which were from 12 to 18 months old. On the other hand, the animals of Group 9,

whose growth was limited by the low cystine content of the diet and which in a period of 10 weeks on the experimental diet were unable to make as great a gain in weight as animals on the stock diet in 2 to 3 weeks (Group 14), have produced hair which was distinctly lower in its cystine content than that produced in the same period of time by the animals fed diets adequate in their contents of cystine as already discussed.

That the lower cystine content of the hair of the animals receiving amounts of cystine insufficient to permit a normal rate of growth was not the result of retardation of growth *per se*, however, is demonstrated by the cystine content of the hair of the rats which also received a diet inadequate for a normal rate of growth, but in which the amount of lysine rather than of cystine in the diet was the limiting factor (Group 13). Although the animals of this group were able to make increments in weight only slightly greater than the animals of Group 9, the hair produced during this period was similar in its cystine content to that of those animals which received amounts of dietary protein and cystine such that marked growth resulted.

The cystine content of the hair of the rats receiving the diet inadequate in its cystine content was similar to and only slightly higher than the cystine content of the hair of the young rats (Group 15), animals whose hair was obtained for analysis before the development of the adult type of hair (7). The animals of Group 14 from which hair was collected during the period of transition from the "puppy" coat to the adult type, and which were fed the stock diet for 2 to 3 weeks only also showed a lower content of cystine in the hair.

The total sulfur content of the hair showed variations similar to and parallel to the cystine content, since it was possible in this case also to distinguish between two types of hair, the low sulfur type (Groups 9, 14, 15) and a higher sulfur type (Groups 10 to 13, 16). That these differences in sulfur and cystine content were not due to differences in content of moisture, ash, or other constituents, was shown by the analyses for total nitrogen which varied little throughout the series. As a matter of fact, the nitrogen content of the hair of Group 15, which showed the lowest average cystine content of all the groups, would appear to be slightly higher than that of any other group.

From these results, it would seem that the initial coat of hair ("puppy" coat) contains a lower content of cystine than the adult coat;² that during the early period of rapid somatic development, although a heavier coat of hair (adult hair) may be produced (2), the cystine content of the hair does not increase significantly; but that in the later stage of somatic development, in which growth proceeds less rapidly, if adequate amounts of cystine are available in the diet, the cystine content of the hair is increased. During the longer period of time, if the cystine content of the diet is not sufficient for either normal somatic development or normal growth of the hair, the percentage of cystine in the hair changes little from that of the "puppy" coat of hair. Retardation of growth, due to a shortage of dietary factors other than cystine, *e.g.* lysine, does not affect the cystine content of the hair, which resembles that of the adult type of hair.

In the series considered, the animals fed experimental diets were for the most part, *of the same age but of widely different body weights* at the time the hair was collected for analysis. It might be argued that age and body weight were more important factors in determining the cystine content of the hair than were dietary factors. In a second series, the animals were placed on a series of experimental diets, but the hair was collected from each animal when it had reached a body weight of approximately 120 gm. In other words, we were concerned with the analysis of hair produced by animals *of the same body weight but of different ages*. The stage of somatic development corresponding to a body weight of 120 gm. was chosen since, in our colony, this weight in control animals was reached at the age of 6 to 7 weeks, the time at which according to Greenman and Duhring (7) the transformation from "puppy" to adult hair has occurred.

The diets of the second series were similar to the preceding. A control group (Group 17) received the stock diet, a second group (Group 18) received the Sherman-Woods diet in which 16 per cent of casein had been substituted for an equivalent amount of carbo-

² Düring (8) observed that the total sulfur content of the hair of rabbits remained relatively constant until the 3rd month, and that the total sulfur increased slightly thereafter as the animals became older. The changes, 4.00 per cent to 4.65 per cent of total sulfur, are similar to those we have observed in the composition of the hair of rats.

hydrate, a third group (Group 19) received the same diet as Group 18 with the further addition of 0.75 per cent of cystine to insure an excess of cystine in the diet. The rate of growth of these three groups, as shown in Table II, was essentially the same, since all animals of these groups had attained a weight of about 120 gm. at the age of 6 to 7 weeks (*i.e.*, after 2 to 3 weeks on the experimental diets).

Two groups received diets which retarded growth. The animals of Group 20 received the basal Sherman-Woods diet as modified

TABLE II.
Cystine Content of Hair.

Group No.	Diet.	Age.	Final weight, average.	Cystine content of hair of individual animals, per cent.	Average for group.
		<i>wks.</i>	<i>gm.</i>		
17	Stock diet.	7	122.8	11.40, 11.44, 12.26, 12.52, 11.38, 12.41	11.90
18	Casein, 16.0 per cent.	6-7	125.0	13.51, 13.82, 15.44, 15.28, 14.29, 14.07, 15.03	14.49
19	Casein, 16.0 per cent; cystine, 0.75 per cent.	6-7	121.4	14.06, 15.14, 14.29, 14.80, 14.51, 15.16, 14.76	14.67
20	No added casein.	11-15	119.0	10.08, 10.05, 10.77, 9.85, 10.93, 10.72, 10.48	10.41
21	Low lysine.	12-14	123.8	12.51, 12.46, 12.75, 12.09, 12.50, 12.46, 12.20	12.42

(2) with no supplementary casein. For the low lysine diet of this series, the gliadin diet of Osborne and Mendel (9) was used. This diet contained gliadin, 18 per cent; salt mixture, 4.5 per cent; dextrin, 50.0 per cent; lard, 24.5 per cent; and cod liver oil, 3.0 per cent.³ On these two diets growth proceeded slowly and an average body weight of 120 gm. was reached at the age of 11 to 15 weeks (*i.e.*, after 7 to 11 weeks on the experimental diets).

The analytical data for this series demonstrate even more strik-

³ Vitamin B was supplied by Vegex in amounts of about 75 mg. daily per rat.

ingly the influence of the cystine content of the diet on the composition of the hair (Table II). The animals of Groups 18 and 19, which were supplied with amounts of cystine fully adequate for or in excess of the requirements for rapid growth, produced hair which contained a high percentage of cystine. There was no significant difference in the composition of the hair of those animals which received excess cystine in the diet (Group 19), thus demonstrating that amounts of cystine in excess of those necessary to meet the demands for growth and to insure the production of hair of normal composition did not result in the production of hair of proportionately greater cystine content. The animals of Group 20, which, in a period of 11 to 15 weeks, *i.e.* nearly twice the time required by the animals of Groups 18 and 19, had reached a body weight of about 120 gm. and which as shown previously had been able to add little hair to their coat in this period (2), had elaborated a coat of hair much lower in its content of cystine, similar in cystine content to the hair of young animals of the age of 30 days (Table I). The animals of Group 21 which also grew slowly due to a dietary deficiency of lysine and whose diet contained cystine in adequate but not excessive amounts, had produced a hair whose cystine content was significantly greater than that of the animals on a cystine-poor diet, a cystine content similar to but slightly higher than that of the stock diet group (Group 17) which made a normal rate of growth.

These results supplement the data of the first series of experiments (Table I). They indicate that in the period of rapid growth hair of a cystine content similar to that of adults may be produced if excess cystine is present in the diet, and that if an adequate but not excessive amount of cystine is furnished by the diet in this early period of growth, the hair may contain a higher percentage of cystine than that produced by young animals, but that under these circumstances, the cystine content of the hair is not as great as in the adult or in the younger animals which have received an excess of cystine in the diet.

It should be noted that Group 10 (Table I) and Group 18 (Table II), both of which received the same diet showed a considerable difference in the percentage of cystine present in the hair. We are unable to determine whether this is a variation due to individuals, season, or age. The experiments with the groups presented

in Table II were carried out subsequent to those in Table I. The rats of Group 18 were from 6 to 7 weeks of age while those animals of the earlier series were about 14 weeks of age. Experiments designed to study more in detail the effect of these and other factors on the composition of the hair have been planned.

SUMMARY.

1. Further evidence is presented which indicates that the cystine requirements for growth of the body take precedence over the cystine requirements for the production of hair in the white rat.

2. The cystine and sulfur contents of the hair of the white rat were found to vary with the cystine content of the diet and to some extent with the age of the animals.

3. The cystine and sulfur contents of the hair of young rats ("puppy" coat) were significantly lower than those of the hair of rats fed on diets high in their content of cystine.

4. Hair from animals which received a diet in which cystine was the factor limiting growth, resembled in its lower cystine and sulfur contents the first coat of hair produced in young rats.

5. Retardation of growth alone did not produce a hair low in its cystine content, since a diet deficient in some other factor than cystine, *e.g.* lysine, did not result in the production of a hair of abnormally low content of cystine or sulfur.

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